J Clin Periodontol 2005; 32 (Suppl. 6): 87–107

Journal of Clinical Periodontology

Aspects of adaptive host response in periodontitis

Tord Berglundh and Mauro Donati

Department of Periodontology, Institute of Odontology, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden

Berglundh T, Donati M. Aspects of adaptive host response in periodontitis. J Clin Periodontol 2005; 32 (Suppl. 6): 87–107. © Blackwell Munksgaard 2005.

Abstract

Objective: To review host response in periodontitis with respect to cellular composition of lesions, T cell receptor (TCR) gene expression, cytokine profiles of T-helper (Th) cells and autoimmune components.

Material and methods: The studies included were confined to human material (biopsies, gingival crevicular fluid, blood from subjects with periodontitis).

Results and conclusions: In periodontitis lesions, plasma cells are the most common cell type and represent about 50% of all cells, while B cells comprise about 18%. The proportion of B cells is larger than that of T cells and Th cells occur in larger numbers than T cytotoxic cells. Polymorphonuclear cells and macrophages are found in fractions of less than 5% of all cells. Lesions in aggressive and chronic forms of periodontitis exhibit similar cellular composition. Differences in disease severity, however, may reflect increases in plasma cell and B cell densities. B cells serve as important antigen-presenting cells in periodontitis. The periodontitis lesion expresses a unique TCR gene repertoire that is different from that in blood. The role of superantigens in periodontitis is unclear. There are few studies using comparative designs and unbiased quantitative methods regarding Th-1 and Th-2 cells in periodontitis. The relative dominance of B cells and plasma cells in periodontitis lesions cannot entirely be explained by enhanced Th-2 functions but maybe because of an imbalance between Th-1 and Th-2. Autoimmune reactions are evident in periodontitis lesions. The role of auto-antibodies in the regulation of host response in periodontitis, however, needs to be clarified. Auto-reactive B cells occur in larger proportions in subjects with periodontitis than in healthy controls.

Key words: periodontitis; pathogenesis; immune response; review

Accepted for publication 1 April 2005

Mechanisms of host response in the periodontal tissues are complex and involve numerous systems of interactions. While the innate host response is characterized by non-specific reactions, the adaptive response utilizes strategies of recognition, memory and binding to support the effector systems in the elimination of challenging elements. While there is no doubt that the innate response is fundamental in periodontitis and is involved in the regulation of adaptive immune response, it is nevertheless covered elsewhere (Madianos et al. 2005, Shapira et al. 2005).

Parts of the adaptive host response in periodontitis are outlined in Fig. 1. In this schematic drawing different areas may be recognized; (i) the nature of the lymphocyte type (T and B cells), (ii) antigen recognition by T cell receptors (TCRs), (iii) cytokine profiles of T helper (Th) cells and (iv) autoimmune reactions that may influence the adaptive host response in periodontitis. In the current review these different areas are discussed. The studies included in the review are confined to human material (gingival biopsies, gingival crevicular fluid (GCF), peripheral blood) sampled from subjects with periodontitis. Main search terms included ''*periodontitis*'', ''*periodontal disease*''. Specific search terms were added for each section reviewed.

Phenotypic Characteristics of Inflammatory Cells in Periodontitis Lesions

The periodontitis lesion is characterized by large proportions of inflammatory cells and vascular structures. Studies, in which the morphological and phenotypic characteristics of the inflammatory cells are described, are reported in Table 1. The specific search terms applied in this section were ''*biopsy, cells, phenotype, histopathology, immunohistochemistry, inflammatory cells, and lesion*''.

Although the criteria for the diagnosis periodontitis and techniques used for the cell detection vary between studies, it is evident that plasma cells and lympho-

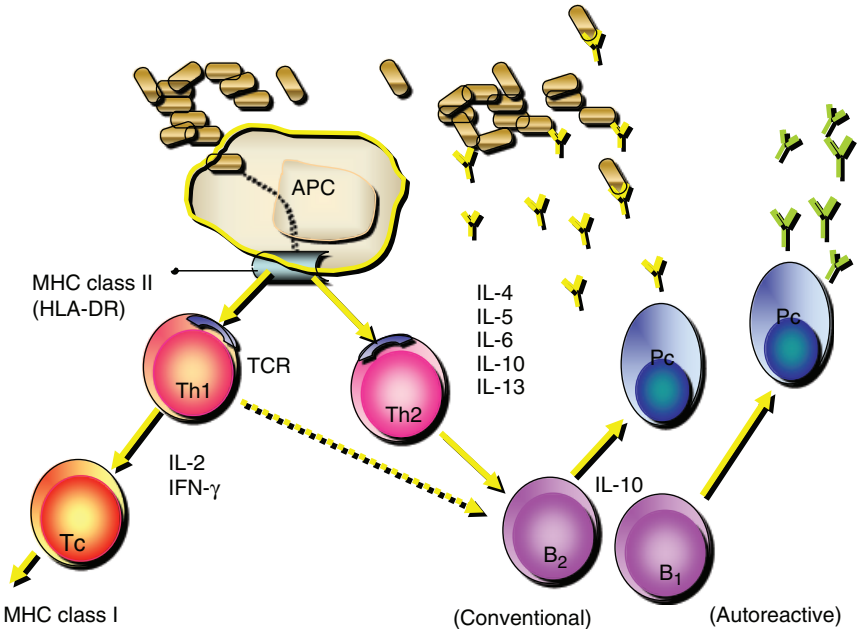

Fig. 1. Schematic outline of regulatory components of adaptive host response in periodontitis.

cytes dominate among cells in the lesions. Lindhe et al. (1980) applied stereological techniques and morphological criteria of cells to assess the composition of inflammatory infiltrates in gingival tissues obtained from 22 subjects with advanced periodontitis. It was reported that plasma cells occupied 31% of the lesion volume, while the proportion of lymphocytes varied between 5% and 10%. Macrophages and polymorphonuclear (PMN) cells were found in densities of 1–2% and fibroblasts in 5%. In other words, the volume occupied by plasma cells was three times larger than the proportion of lymphocytes. Other inflammatory cells occurred only in small numbers. Similar findings were reported from studies in which morphological/stereological techniques were utilized (Liljenberg & Lindhe 1980, Passo et al. 1988, Zappa et al. 1991, Liljenberg et al. 1994, Berglundh et al. 1998).

The majority of studies describing cellular characteristics of periodontitis have applied immuno-fluorescence or immunohistochemical techniques. Early reports distinguished lymphocytes into T and B cells, while later studies included also the various T cell subpopulations. Mackler et al. (1977) collected gingival tissues from five subjects and reported that specimens representing periodontitis, in contrast to healthy or gingivitis samples, contained substantial numbers of immunoglobulin (IgG, IgM)-bearing lymphocytes and plasma cells. Seymour & Greenspan (1979) analysed 12 biopsies of patients with chronic periodontitis (CP) and found that the majority of lymphocytes in the lesions had the B cell phenotype and were positive for IgM and IgG. Charon et al. (1981) and Okada et al. (1983) analysed gingival biopsies from patients with advanced periodontitis. It was reported that plasma cells dominated the lesions, that T cells and macrophages were present, while PMN cells were few. In a study comparing suppurating and non-suppurating periodontitis lesions, Passo et al. (1988) found that in both types of lesions plasma cells and lymphocytes predominated. The authors stated that the majority of lymphocytes were B cells and, within the group of T cells, Th cells outnumbered T cytotoxic cells. Cobb et al. (1989) compared inflammatory cell infiltrates obtained from CP, gingivitis and healthy gingival tissues. T and B lymphocytes occurred in larger numbers in diseased than in healthy sites, while only small differences were found between the periodontitis and healthy tissue specimens regarding the number of NK cells. The findings that B cells occur in larger numbers than T cells in periodontitis lesions are not consistent in all studies. Mödeer et al. (1990) analysed biopsies obtained from adolescents with early signs of periodontitis and reported that T cells dominated in the inflammatory cell infiltrates. Berglundh et al. (1998) found similar proportions of T and B cells in gingival biopsies obtained from 21 subjects with advanced periodontitis.

Progress in the development of immunohistochemical markers made it possible to obtain more detailed information of lymphocytes in periodontitis lesions, and, hence, phenotypic characterization of cells was performed parallel to assessments of additional features of the cells such as receptor and memory functions (Gemmell et al. 1992, 2001, Yamazaki et al. 1993, Lappin et al. 1999). Yamazaki et al. (1993) reported that Th cells (CD4+) in periodontitis lesions frequently exhibited memory (CD45RO+) characteristics and that B cells to a varying extent were positive to early (CD23+) or late (CD25+) markers of activation.

Antigen-presenting cells (APCs) in periodontitis lesions

Several cells serve as APCs. Langerhans cells, macrophages and dendritic cells are professional APCs and contribute to antigen recognition and early response mechanisms in host defence. B cells are also capable of serving as APCs and express class II antigens upon stimulation and use the capacity of their memory systems in antigen presentation. Co-stimulatory molecules associated with APC–TCR interactions in periodontitis were investigated by Gemmell et al. (2001). It was reported that the proportion of T cells positive for the co-stimulatory signal receptor (CD28+) increased with increasing percentage of B cells. Further, cells expressing the natural ligand B7-2 (CD86+) occurred in larger numbers than cells with B7-1 (CD80+). This may indicate that there is a more pronounced Th2 than Th1 response in periodontitis. Orima et al. (1999) in a similar study reported on the presence of not only CD80+ and CD86+ cells, but also the co-stimulatory molecules, CD40 and CD40L. Mahanonda et al. (2002) observed a significant up-regulation of CD86 and the dendritic cell-marker CD83 on B cells in periodontitis lesions. The authors suggested that B cells may serve as potent APCs in the immune response of periodontal disease. The role of B cells as APCs was also described in

study on gingival biopsies obtained from 25 subjects with periodontitis (Gemmell et al. 2002). In the largest lesions, the B cell group was the predominant type of APCs. It was suggested that B cell antigen presentation may allow further activation and clonal expansion of already activated T cells.

Progressive lesions

Attempts were also made to characterize ''active'' periodontitis lesions, i.e. sites that exhibit a recent history of attachment loss. Zappa et al. (1991) found larger proportions of plasma cells, lymphocytes and total numbers of inflammatory cells in gingival specimens prepared from ''progressive'' sites than in sites with no history of attachment loss. Different results were reported by Reinhardt et al. (1988), who classified sites that demonstrated attachment loss $\geqslant 2$ mm within the past 3 months as ''active''. Soft-tissue biopsies from ''active'' and stable periodontitis sites as well as from healthy sites were collected. It was reported that the ''active'' lesions had larger proportions of different T cell subpopulations than healthy tissues, while B cells were more prevalent in ''active'' than in stable sites. Liljenberg et al. (1994) compared periodontitis lesions collected from active sites (history of attachment loss >2 mm at three sites or more) and inactive sites in a group of eight subjects with advanced periodontitis. Biopsies were also obtained from diseased sites in a group of 11 subjects who had no signs of attachment loss. Both T and B cells were found in larger proportions in active than in inactive sites. No differences were found between the lesions from the inactive sites of the eight subjects and the specimens produced from the 11 subjects with no attachment loss. In this context it is interesting to note that in studies evaluating periodontitis lesions before and after non-surgical therapy, both T- and B-cell densities declined after treatment (Berglundh et al. 1999, Kleinfelder et al. 2001).

Aggressive periodontitis

The above reported findings were based on analysis of samples obtained from subjects with adult/chronic periodontitis (AP). Several studies described tissues from other forms of periodontitis, e.g. early onset (aggressive) periodontitis (EOP) including juvenile (JP) and prepubertal (PP) forms and rapidly progressive periodontitis (RPP). In a comparative study on JP and AP lesions, Liljenberg & Lindhe (1980) reported that plasma cells dominated in both types of infiltrates and that the proportion of lymphocytes was significantly larger in the specimens representing AP. In a similar type of investigation, Gillett et al. (1986) stated that more than 50% of cells in JP lesions were plasma cells. The cellular composition in AP, however, varied considerably between subjects and plasma cells represented about 30% of cells. Joachim et al. (1990) in a study on JP and AP lesions also identified plasma cells in large numbers and observed that the cell count increased with disease severity. The authors further reported that signs of degeneration of plasma cells were more conspicuous in sections from JP than from AP. Kleinfelder et al. (2001), who used different markers to identify plasma cells, B cells and memory T cells, failed to demonstrate differences in cell composition in adult periodontitis and EOP lesions. Lappin et al. (1999), however, reported that the proportion of B cells was larger and the T cell percentage smaller in AP than in EOP sites. In addition, Berglundh et al. (2001) compared the composition of gingival lesions from 21 subjects with AP and six children with localized aggressive periodontitis (LAP). Stereological assessments and immunohistochemical analysis revealed that lymphocytes and B cells in particular, occupied significantly larger volume fractions in LAP than in AP lesions.

Some features of the previously recognized disease entity RPP were also investigated. Çelenligil et al. (1993) analysed gingival specimens from 16 subjects with RPP. IgG-positive plasma cells dominated and Th (CD4+) and T cytotoxic (CD8+) cells occurred in almost similar numbers. Hillmann et al. (2001) studied RPP in relation to AP. They collected gingival biopsies from 10 RPP patients and five AP patients and reported that both types of specimens contained large proportions of plasma cells and B cells and that the total number of inflammatory cells was higher in RPP than in AP. It was concluded that the inflammatory process regarding number of inflammatory cells and the amount of tissue destruction was stronger in RPP than in AP.

Summary

The reported findings regarding different cell proportions in AP are virtually consistent, while larger differences are found in studies on aggressive forms of periodontitis. The overall distribution of inflammatory cells in periodontitis lesions is presented in Fig. 2. Plasma cells are the most common cell type and represent about 50% of cells, while B cells comprise about 18%. The proportion of B cells is larger than that of all T cells and Th cells occur in larger numbers than T cytotoxic cells. PMN cells and macrophages are found in fractions of less than 5% of all cells.

Lesions in aggressive and chronic forms of periodontitis exhibit similar features with respect to cellular composition. Differences in disease severity, however, may in both forms of periodontitis affect plasma cell and B cell densities.

Both T cells and B cells express costimulatory molecules and contribute to antigen recognition and cell activation. B cells serve as important APCs in periodontitis.

TCRs

Host response to antigenic challenges in periodontitis includes specific immune reactions in which the TCR interacts with the processed antigen of APCs. The TCR consists of two polypeptide chains including variable (V) segments

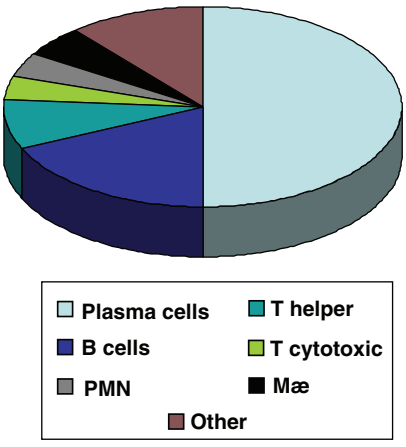

Fig. 2. Distribution of cell proportions in periodontitis lesions calculated from studies reported in Table 1. Plasma cells, B cells, T helper, T cytotoxic, polymorphonuclear (PMN) cells, macrophages (Mϕ) and other cells (e.g. mast cells, fibroblasts, unidentified cells).

Table 1. Phenotypic characteristics of cells in periodontitis lesions

Reference	Periodontal diagnosis	Sample	Technique	Results
Mackler et al. (1977)	Five patients of various age and sex	Gingival biopsies clinically normal, mild gingivitis, periodontitis	Immunofluorescence	Higher concentration of lymphocytes and plasma cells (IgG and IgM) in periodontitis biopsies compared with normal healthy biopsies
Seymour & Greenspan (1979)	Patients with chronic periodontal disease	Twelve biopsies. Sites scheduled for surgery with PPD 4–8 mm and BoP+	Immunohistochemistry Immunofluorescence	Majority of the lymphocytes had the phenotype of B cells and were positive for IgM and IgG
Lindhe et al. (1980)	Twenty-two patients with advanced periodontal tissue destruction, with PPD8 and 50% bone loss	In each patient, six sites were selected representing advanced disease, established gingivitis and ''healthy'' gingiva	Morphometric analysis. For each biopsy numerical density (Nv) and volumetric density (Vv) of cells were calculated	Periodontitis lesion: 31% plasma cells, 5–10% lymphocytes, 5% fibroblast, 1.3% macrophages, 1.3 Neutrophils G., 11% collagen. In the gingivitis lesion the ratio lymphocytes-plasma cells was 1:1, in the periodontitis was 1:3
Liljenberg & Lindhe (1980)	Eight juvenile periodontitis, seven post-juvenile periodontitis, seven adult periodontitis	Biopsies from diseased sites with PPD >8 mm, and >50% of bone lost	Morphometric analysis	The ICT of all gingival units from the three categories of patients was characterized by high plasma cell density; between 56.8% and 84% of all cells were Plasma cells. Proportion of lymphocytes was small in juvenile and post-juvenile sections (2.6 n., 0.9 v.) but larger in the adult periodontitis group
Charon et al. (1981)	Fifteen patients 30–88 year-old with advanced periodontal disease	Biopsies from both diseased and healthy sites and blood samples from each patient	Immunohistochemistry	The plasma cell dominated in the periodontitis lesion. The presence of T cells and activated macrophages indicated that both humoral and cell-mediated responses are operative in human chronic periodontitis
Okada et al. (1983)	Patients with advanced periodontitis (29–55 years of age)	Biopsies from sites with PPD$\geqslant$5 mm and evidence of bone destruction	Immunohistochemistry	Only few PMNs were observed. Plasma cells predominated in the central portion of the lamina propria, with the proportions positive for IgG, IgA and IgM accounting for 65.2% 11.2% and 1.3% of the total infiltrating cells
Gillett et al. (1986)	Three groups: childhood gingivitis, juvenile periodontitis, chronic adult periodontitis	Two biopsies childhood gingivitis, 12 chronic adult periodontitis, six juvenile periodontitis	Immunohistochemistry, monoclonal antibody-HLADr	In juvenile periodontitis biopsies, $>$50% of the cells were plasma cells. Lesions in chronic adult periodontitis were dominated by lymphocytes HLADr+(B cells) and plasma cells
Passo et al. (1988)	Advanced chronic periodontitis patients (9)	Thirty-three bleeding suppurating (S) and 23 bleeding non-suppurating (NS) inter-proximal biopsies	Immunohistochemistry and morphometric analysis	In both suppurating (S) and non-suppurating (NS) biopsies, plasma cells and lymphocytes dominated. The vast majority of T cells were of T helper, with few T cytotoxic/suppressor cells
Reinhardt et al. (1988)	Thirteen adult periodontal maintenance patients	Biopsies from ''active'' sites ($\geqslant$2 mm clinical attachment loss within 3 months of biopsy) and clinically similar but ''stable'' or healthy sites	Immunohistochemistry, monoclonal antibodies for (1) pan T cells, (2) T cytotoxic/suppressor cells, (3) T helper/inducer cells and (4) pan B cells	Pan B cells were significantly more prevalent in infiltrates from active sites than in stable ($p<0.05$) or healthy ($p<0.01$) sites. The T/B cell ratio was also significantly lower in active than stable biopsies ($p<0.05$)
Cobb et al. (1989)	Twenty-four patients divided in three groups: healthy gingiva, chronic	Healthy gingiva: PI = 0, GI = 0, chronic gingivitis: PI$<$1.0, GI at least 2.0, gingival sulcus 0–3 mm;	Immunohistochemistry. monoclonal antibodies for T lymphocytes (UCHL-1) B lymphocytes (CD-45R),	The T- and B-lymphocyte populations increased approximately 20 $\times$ progressing from healthy to gingivitis to periodontitis specimens, the NK-cell population showed only a 3 $\times$ increase which represented

	gingivitis and chronic adult periodontitis	chronic periodontitis: PI < 1.0, GI at least 2.0, PPD ⩾ 6 mm	and NK cells (Leu-7 and Leu-11b)	19%, 6.6% and 7% of the total of all positively stained lymphocytes across biopsy groups
Joachim et al. (1990)	Biopsies from 20 patients and three control volunteers	Five with treated adult periodontitis (AP), five with untreated AP, five with treated juvenile (JP) and five with untreated JP.	Electron microscopy and quantification of plasma cells by ultra-structural classification at magnification of × 1400 and × 5000	Plasma cell (PC) counts increased significantly with lesion severity. Degenerated PC tended to be more numerous within JP tissue when compared with AP
Modeer et al. (1990)	Gingival biopsy specimens from adolescents ($n = 10$). Specimens from gingivitis lesions ($n = 5$) were used as controls	Biopsies from diseased sites were retrieved from periodontal pockets with an increased probing depth greater than 5 mm.	The mononuclear cell populations in the specimens were detected by using monoclonal antibodies defining functional T lymphocyte subpopulations, B lymphocytes, and monocytes	All gingival specimens from patients with increased probing depth showed large lymphocyte infiltrates, most of which were CD3 positive cells (T lymphocytes). B cells were detected in most specimens from the periodontitis group and varied from less than 1–21%
Zappa et al. (1991)	Ten adult patients with untreated advanced periodontitis were monitored during a period of 10 months	Using an electronic pressure sensitive probe sites were identified which had ⩾ 2 mm attachment loss within the previous month (P) and non-progressive sites (C)	The cells were identified and quantified by light microscopic evaluation of nuclear and cytoplasmatic staining at a magnification of × 1250	In P-sites, the numbers of macrophages, plasma cells, lymphocytes and total inflammatory cells were significantly higher as compared with C-sites. There were no differences in cell populations between superficial and deep connective tissue areas within P- and C-sites
Gemmell et al. (1992)	Six healthy/gingivitis subjects and 26 adult periodontitis patients (AP)	Peripheral blood and gingival biopsies	Monoclonal antibodies, Two-colour immunofluorescence, Flow cytometry.	The proportion CD45RA+CD4 cells were 9% in healthy tissue and 22% in AP. In peripheral blood, a lower percentage of CD4 cells were CD45RO+ and CD45RA+ compared with gingival biopsies
Celenligil et al. (1993)	Patients (16) with rapidly progressive periodontitis (22–33 years of age)	Gingival biopsies from the sites advanced bone destruction, (PPD ⩾ 7 mm)	Histopathology and immunohistochemistry analysis	The majority of mononuclear cells were IgG+plasma cells, which dominated lesion with equal participation of both T cell subpopulations
Yamazaki et al. (1993)	Patients with moderate to advanced adult periodontitis (19) and gingivitis control patients (5).	Periodontitis biopsies with varying degree of inflammation (GI of 0–2) PPD > 4 mm and CAL > 5 mm. Gingivitis specimens had (GI of 1, PPD and CAL ⩽ 3 mm)	Immunohistochemistry. A double staining technique was used to identify CD4+, CD45RO+memory T cells and activated CD19+B cells expressing CD23 or CD25	The percentage of CD23+ and CD25+, CD19+B cells, which were identified in 13 out of 19 samples from periodontitis, varied significantly in spite of similar clinical status. The frequency of B cells in the gingivitis was much lower than that of periodontitis 15.0 ± 5.4 *versus* 53.0 ± 24.2 (B cells)
Liljenberg et al. (1994)	Eight subjects (test) with advanced periodontal disease, with > 2 mm of attachment loss at ⩾ 3 sites in a 12-month interval and 11 subjects (control) with non-progressive sites	Test subjects; biopsies from progressive disease active (PDA) and progressive disease inactive (PDI) sites. Eleven biopsies from patients with non-progressive disease (NPD)	Morphometric analysis of the ICT and immunohistochemical examination by monoclonal antibody towards CD3, CD4, CD8, CD14, CD19 and CD22. The relative volume of positively stained cells in the ICT was determined by microscope and double grid 16/400	The progressive disease sites (PD) were comprised of a larger volume of plasma cells, a higher percentage number of macrophages and lower numerical density of lymphocytes than the (NPD) group. Both T cell markers (CD3 and CD4) and B cells markers (CD22) were significantly elevated in the PDA compared with the PDI lesions

Table 1. (Contd.)

Reference	Periodontal diagnosis	Sample	Technique	Results
Berglundh et al. (1998)	Advanced adult periodontitis (21 subjects) healthy controls (16)	Gingival biopsies from AP and PBMC from both the groups	Immunohistochemistry Flow cytometry	Similar proportions of T and B cells in gingival biopsies obtained from 21 subjects with advanced periodontitis
Orima et al. (1999)	Fourteen patients with moderate to advanced adult periodontitis	Sixteen biopsies PPD = 7.8 ± 2.8; CAL = 9.6 ± 2.6; BL(%) = 84.7 ± 16.3; Bop(*n*) = 14/16	Immunohistochemistry to detect the expression of CD40, CD40 ligand (CD40L), CD80, CD86, CD28 and cytolytic T lymphocyte-associated antigen-4 (CTLA-4) on lymphocytes	While most T cells and B cells expressed CD28, and CD80 and CD86 in gingival tissues, the expression of CD40L and CTLA-4 was lower and highly variable between specimens. The distribution of CD40+ cells was similar to that of CD19+ cells. The percentage of CD40+ cells in the CD19+ cells was nearly 100%
Berglundh et al. (1999)	Sixteen individuals with advanced periodontal.	Biopsies before and at 12 and 24 months after periodontal therapy (SRP). Peripheral blood obtained from the subjects at the 24-month re-examination	Immunohistochemical analysis and flow cytometry	Improved clinical condition following SRP, was accompanied by a substantial reduction of the size of the ICT. Following therapy the densities of CD19 and CD3 positive cells and cells expressing T cell receptor V β genes were reduced in the ICT. But the relative distribution of lymphocyte subsets in peripheral blood was unchanged
Lappin et al. (1999)	Nine patients with adult periodontitis (AP), from 10 patients with early onset periodontitis (EOP)	Biopsies form AP (9), EOP (10) and gingival healthy tissues (4) from AP patients	Immunohistochemistry. Monoclonal antibodies to CD 20 (B cells), CD 3 (T cells) and CD 45RO (memory T cells), CD 4 (T helper) CD 8 (T suppressor) and CD 68 (monocyte/macrophage)	A greater number of B cells were observed in the diseased than in the healthy tissues. There was a significant increase in the B to T cell ratio in AP compared with EOP and gingival healthy sections. The proportion of T cells was lower in the AP than in the EOP sections
Orima et al. (1999)	Fourteen patients with moderate to advanced adult periodontitis (AP)	Gingival biopsies. Mean probing pocket depth (PPD) of the biopsy sites: 7.8 ± 2.8	Immunohistochemistry. Monoclonal anti-CD3 and anti-CD19 were used for double staining by combining methods to identify T cells and B cells	In CD3/CD19 double-stained sections, CD19+ cells were the dominant infiltrating cell type
Berglundh et al. (2001)	Eleven children (9.5 ± 2.0 years) with periodontitis (LPP group) and 21 adults with advanced adult periodontitis (AP group)	Gingival biopsies and peripheral blood sample obtained from all in the AP group and from seven of the LPP group	Morphometric and immunohistochemical analysis	The ICT in the biopsies of the LPP group contained a larger proportion of lymphocytes and, in particular B cells, than was the case in the AP group. The content of lymphocyte subpopulations in peripheral blood in the two groups was almost similar

Gemmell et al. (2001)	Twenty-one healthy/ gingivitis individuals and 26 periodontitis subjects	Biopsies from healthy (PPD <4 mm) and from moderate to advanced disease (PPD >4 mm). The samples were grouped according to the size of the infiltrate	An immunoperoxidase technique was used to examine co stimulatory molecules CD28, CD152, CD80 and CD86 in T and B lymphocytes and macrophages in gingival biopsies	A higher percentage of CD86+ cells indicated predominance of Th-2 response in both healthy/gingivitis and periodontitis tissues. The analysis of revealed that CD80 was expressed predominantly by macrophages while both macrophages and B cells expressed CD86
Hillmann et al. (2001)	Ten patients with rapidly progressive periodontitis (RPP) and five patients with adult periodontitis (AP)	Biopsies from sites with PPD 8–12 mm and alveolar bone loss $\geqslant 50\%$	Immunohistological methods	Both type of specimens contained large proportions of plasma cells and B cells and the total amount of chronic inflammatory cells was higher in RPP than in AP
Gemmell et al. (2001)	Twenty-one healthy or gingivitis subjects and 25 periodontitis patients	Gingival biopsies. The samples were grouped according to the size of the lesion: group 1, small; group 2, medium; group 3, extensive infiltrates	An immunoperoxidase technique was used to investigate CD1a+, CMRF-44+, CMRF-58+ and CD83+dendritic cells, CD14+, CD19+B cells, macrophages and dendritic cell precursors	B cells were the predominant APC in group 2 and 3 tissues. The percentage of B cells in group 3 lesions was increased in comparison with group 1 healthy tissues and also in comparison with group 3 healthy/gingivitis sections
Kleinfelder et al. (2001)	Ten subjects with early-onset periodontitis with Aa (EOP-Aa) and 10 subjects without Aa (EOP-nonAa)	Two biopsies at baseline and two biopsies after a full-mouth scaling and root planing	Immunohistological analysis: monoclonal antibodies to CD20 (B cells), CD30 (plasma cells) and CD45RO (T memory cells)	CD30+ cells (Plasma cells) were in the highest number compared with any other phenotype in both groups. Significant change in CD30+ cells before and after therapy occurred in the EOP-Aa group
Gemmell et al. (2002)	Twenty-one subjects healthy/gingivitis and 25 patients with moderate to advanced periodontitis	Biopsies from each subject during periodontal surgery for disease or non-disease related reasons. The samples were grouped according to the size of the lesion: group 1, small; group 2, medium; group 3, extensive infiltrates	Cell analysis for quantitative assessment of the size of the ICT. An immunoperoxidase technique to investigate the presence of CD1a+, CMRF-44+, CMRF-58+and CD83+dendritic cells, CD14+macrophages or dendritic cell precursors and CD19+B cells	Numerous CD1a+Langerhans cells in the epithelium in all groups. The percentage of CD83+dendritic cells in the ICT was higher than the percentage of CD1a+, CMRF-44+ or CMRF-58+ dendritic cells. The percentage of CD14+ cells in the inflammatory infiltrates was similar to that of CD83+ cells. B cells were the predominant APC in group 2 and 3 tissues. The percentage of B cells in group 3 periodontitis lesions was increased in comparison with group 1 periodontitis tissues and also in comparison with group 3 healthy/gingivitis sections
Mahanonda et al (2002)	Six patients with generalized severe adult periodontitis	Biopsies at sites with $>2/3$ of the root length of bone loss, mobility degree III	Flow cytometry. Monoclonal antibodies towards CD19, CD14 CD56, CD80, CD86 and CD83	Significant upregulation of CD86 and CD83 expression was detected in periodontitis lesions, and most of this occurred on B cells. Analysis of APC function by bacterial activation revealed that B cells served as potent APCs in mixed leucocyte reactions and stimulated T cells to produce high levels of γ interferon

resembling those in Fab fragments of immunoglobulins. While the major part (>95%) of T cells in peripheral blood exhibit the TCR V α and β chain, the remaining fraction contains the γ/δ type. Although the γ/δ type has been identified in periodontitis, it is highly likely that the bulk of antigen recognition is through the α/β TCR. Further, the α/βTCR, in contrast to the γ/δ type, can recognize processed antigens in conjunction with the class II molecule of APCs. Alternatively, a distinct group of antigens, called super-antigens, binds to the outer surface of the class II molecule and the V β gene of the TCR. Different microorganisms produce super-antigens and via the binding to certain parts of the V β gene substantial proportions of T cells may be activated. The role of super-antigens in relation to periodontitis is unclear and conflicting results have been presented (Zadeh & Kreutzer 1996, Karimzadeh et al. 1999, Yamazaki et al. 2000, Gao &Teng 2002).

Different expressions of the TCR V α/β phenotype have been observed in autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis (RA) (Oksenberg et al. 1990, Sottini et al. 1991, Bröker et al. 1993) and in oral diseases like oral lichen planus lesions (Simark-Mattsson et al. 1994). A biased expression of TCR V α/β genes was also demonstrated in periodontal disease and relevant studies are reported in Table 2. Most studies included subjects with AP while aggressive (EOP, PP, JP) forms were included in only a few studies. The specific search terms included *T cell-receptors, TCR, T cells, V α/β phenotype, V α/β gene*.

Nakajima et al. (1996) examined 20 patients with moderate to advanced adult periodontitis and nine subjects with gingivitis with respect to expression of different TCR V α/β genes in gingival tissues and peripheral blood (PBL). It was reported that cells expressing the V β5 gene subfamily and the V β6.7 gene outnumbered the remaining cells in gingival tissues from both the gingivitis and the periodontitis group and that there was a discrepancy between PBL and the gingival tissues regarding proportions of TCR genes. Yamazaki et al. (1996), who applied PCR techniques to determine the occurrence of 22 V β gene families in 14 subjects with adult periodontitis, also reported on a different expression of V β genes in gingival tissue cells and in peripheral blood mononuclear cells (PBMC). The finding that the TCR repertoire is different in periodontis lesions and in peripheral blood of adult periodontitis subjects is consistent in most studies (Geatch et al. 1997, Berglundh et al. 1998, 1999, 2001, Karimzadeh et al. 1999, Ohsawa et al. 2000, Yamazaki et al. 2000, 2001, 2002, Gao & Teng 2002) and indicates that the TCR expression in the periodontitis lesion is unique and involves a restricted number of V βgene families. Zadeh & Kreutzer (1996) in study on gingival biopsies obtained from eight subjects with adult periodontitis, concluded that two or three V β gene families account for all T cells in periodontitis lesions. Thus, Geatch et al. (1997) in a study on 24 periodontitis patients restricted their DNA sequencing analysis to the V β2, V β6 and V β8 genes and Karimzadeh et al. (1999) in a similar study, reported that T cells in periodontitis lesions exhibited larger fractions of V β5.2, V β6 and V β9. On the other hand, (Gemmell, et al. 1997) found that with the exception of the V β13 gene, several of V β3, V β5 and V β6 gene families occurred in larger proportions in cells from gingivitis/healthy tissues than in cells from periodontitis sites.

A different distribution of TCR V βgenes in periodontitis lesions was described by Berglundh et al. (1998). They analysed gingival biopsies and PBMC from 21 subjects with advanced adult periodontitis and found that the V β17 was the most frequent TCR marker in the lesions followed by V β5, V β8 and V β13 gene families. In addition, the TCR V α/β expression of PBMC in the periodontitis subjects was similar to that in healthy controls. In a subsequent study from the same group (Berglundh et al. 1999) changes in the TCR repertoire following non-surgical periodontal therapy were analysed. It was demonstrated that between baseline and 2 years following therapy there was marked decrease in TCR expression in gingival lesions, while no changes in the distribution of TCR genes occurred within PBMC.

Further characterization of TCR genes in periodontitis revealed sequence heterogeneity within V β genes (Geatch et al. 1997) as well as selective expansion of T cells in gingival lesions (Yamazaki et al. 2000). The latter finding was interpreted to indicate that infiltrating T cells recognize a limited number of antigens in periodontitis. Additional support for a role of infiltrating T cell clones in periodontitis lesions was presented by Yamazaki et al. (2002). They isolated T cells from gingival biopsies and PBMC of 16 subjects with moderate to severe CP and from PBMC obtained from 10 healthy controls. It was reported that the proliferative response to heat-shock protein 60s (hsp60s) of PBMC T cells was higher in periodontitis patients than in controls. Nucleotide analysis of TCR genes demonstrated similar expression in gingival T cells and in PBMC of periodontitis patients. This finding suggests that hsp60s-reactive T cells occur in periodontitis lesions and, thus, contribute to autoimmune reactions. In this context the finding that also NK-T cells exhibit certain TCR markers is interesting. Yamazaki et al. (2001) reported that periodontitis lesions contain larger proportions of V α24 NK-T cells than gingivitis lesions. The authors suggested that V α 24 NK-T cells are recruited in order to down-regulate autoimmune reactions in periodontitis.

The variation in expression of V β genes in periodontitis lesions has also been related to differences in the composition of the subgingival microflora. Mathur et al. (1995) isolated PBMC T cells from 12 patients with EOP and 11 healthy controls. Co-culture with *Prevotella intermedia* resulted in up-regulated expression of V αβ2, V β5 and V β6, and it was suggested that periodontitis-associated bacteria may influence TCR V α/β expression in periodontitis. A similar explanation was suggested by Berglundh et al. (2001), who compared the local and systemic TCR V α/β expression in 11 subjects with localized aggressive periodontitis and in 21 subjects with CP. It was reported that no differences were observed regarding the TCR repertoire in PBMC, while a different distribution of V α/β genes was detected in the periodontitis lesions. In comprehensive analysis on TCR genes in four patients with localized JP (LJP), Gao & Teng (2002) reported that no differences between subjects was seen regarding the TCR expression of PBMC. In T cells from gingival tissues, however, few dominant genes of V α/β genes were shared. It was suggested that T cells in LJP patients respond to a limited number of antigens.

Summary

TCR V α/β expression in peripheral blood samples of subjects with periodontitis does not differ from that of

Table 2. T cell receptor (TCR) α/β gene expression in periodontitis

Reference	Periodontal diagnosis	Sample	Technique	Results
Mathur et al. (1995)	Early-onset periodontitis (12) Healthy controls (11)	Peripheral blood mononuclear cells (PBMC)	In vitro; immunohisto-chemistry, co-culture with bacteria and unstimulated expression	Unstimulated expression V α 2, V β 2 and V β 8 higher in patients than in controls. Co-culture with *Prevotella intermedia* upregulated expression for all 5 V β families studied in both patients and controls
Nakajima et al. (1996)	Moderate to advanced adult periodontitis (18 subjects) Gingivitis (9?)	Gingival biopsies (20+9) PBMC (10 periodontitis and two gingivitis)	Immunohistochemistry Flow cytometry	V β5 subfamily and V β6.7 dominated in biopsies from both periodontitis and gingivitis. V β8 was higher and V β5.1 was lower in periodontitis than in gingivitis. TCR gene expression different in peripheral blood and in gingival tissues of periodontitis patients
Yamazaki et al. (1996)	Moderate to advanced adult periodontitis (14 subjects)	Gingival biopsies (15) PBMC	RNA extraction and PCR 22 V β primers	Expression of V β 6 was higher and V β 16 was lower in gingival tissues than in PBMC
Zadeh & Kreutzer (1996)	Adult periodontitis (eight subjects) Healthy controls (four subjects)	Gingival biopsies (8+4) PBMC from five healthy controls	Isolation of gingival leucocytes. Immunofluorescence Flow cytometry	Two or 3 V β families account for up to 50% of all T cells from periodontitis lesions, while gingival tissues and PBMC in healthy subjects used V β families more evenly
Geatch et al. (1997)	Chronic adult periodontal disease (24 subjects) Healthy controls (10+6 subjects)	Gingival biopsies (24) PBMC (16 periodontitis, 16 healthy)	Reverse transcriptase-polymerase chain reaction (RT-PCR) on V β2, V β6 and V β8. Sequencing.	Expression of V β2, 6 and 8 varied in the periodontitis group. Control subjects consistently expressed the three V β families in biopsies and PBMC
Gemmell et al. (1997)	Moderate to advanced adult periodontitis (31 subjects) Healthy/gingivitis (HG; 27 subjects)	Gingival biopsies (15) PBMC	Two-colour flow cytometry of isolated gingival mononucl cells. *Porphyromonas gingivalis* stimulated PBMC sequencing	High percentage of V β 13 in several AP sites. Higher percentage of V β5.2–3 CD4 cells and V β5.1- and 5.2–3 CD8 cells in HG than in AP tissues
Berglundh et al. (1998)	Advanced adult periodontitis (21 subjects) Healthy controls (16)	Gingival biopsies from AP PBMC from both groups	Immunohistochemistry Flow cytometry	V β 17 dominated in gingival lesions TCR gene expression in biopsies different from PBMC. No differences in TCR genes in PBMC between test and control subjects
Karimzadeh et al. (1999)	Adult periodontitis (18 subjects)	Gingival biopsies (25) PBMC	Single-cell suspensions of gingival tissues. Three-colour flow cytometry	Higher percentage of V β 5.2, V β 6 and V β 9 in gingival cells than in PBMC. V β 5.2 expression high in gingival CD45RO+ cells and V β14 high in blood CD45RO − cells. The TCR gene repertoire is different in gingival tissues and blood
Berglundh et al. (1999)	Advanced adult periodontitis (16 subjects)	Gingival biopsies and PBMC at baseline and 2 years after therapy.	Immunohistochemsitry Flow cytometry	Marked decrease in TCR expression in gingival biopsies from baseline to follow-up at 12 and 24 months. No change in PBMC from baseline to 24 months.
Ohsawa et al. (2000)	Adult periodontitis (10 subjects)	Gingival biopsies PBMC	V β6-PCR–single-strand conformation polymorphism (SSCP). DNA sequencing	Accumulated clones of V β6 T cells in periodontitis lesions but not in PBMC

Table 2. (Contd.)

Reference	Periodontal diagnosis	Sample	Technique	Results
Yamazaki et al. (2000)	Adult periodontitis (12 subjects)	Gingival biopsies PBMC	RT-PCR–SSCP. DNA sequencing	Differences in clonality between gingival cells and PBMC. Superantigen stimulation of T cells is unlikely in periodontitis lesions
Berglundh et al. (2001)	Localized pre-pubertal periodontitis; 11 subjects) Advanced adult periodontitis; 21 subjects)	Gingival biopsies PBMC (seven LPP, 21 AP)	Immunohistochemistry Flow cytometry	V $\alpha 2$ dominated in LPP and V $\beta 17$ in AP lesions. No differences in the TCR gene expression in PBMC between the two groups
Yamazaki et al. (2001)	Moderate-to-severe chronic periodontitis (15 subjects) Healthy/gingivitis (12)	Gingival biopsies PBMC	RNA separation and PCR Sequence of V $\alpha 24$ Immunohistochemistry V $\alpha 24$, CD1d, CD3	Larger elevation of V α 24 NK T cells in periodontitis lesions than in gingivitis as compared to levels of V α 24 NK T cells in PBMC of both groups
Gao & Teng (2002)	Localized juvenile periodontitis (four subjects) Controls (two subjects)	Gingival biopsies (*Actinobacillus actinomycetemcomitans* associated; 4) PBMC (4+2)	Quantitative PCR on CD4+ cells from biopsies or PBMC	No differences in TCR genes of PBMC between subjects. Most V α and β genes detected in LJP patients and only few dominant genes were shared. *A. Actinomycetemcomitans* is unlikely exhibiting superantigen properties
Yamazaki et al. (2002)	Moderate-to-severe chronic periodontitis (16 subjects) Healthy controls (10)	Gingival biopsies (16 periodontitis) PBMC (16+10)	RNA separation, PCR and SSCP. Stimulation with heat shock protein 60s (hsp60) and *P. gingivalis*	Proliferative response of PBMC following hsp60 stimulation was higher in periodontitis patinets than in controls. Similar TCR genes in periodontitis lesions as in hsp60-reactive clones of patients

healthy individuals. The periodontitis lesion expresses a unique TCR repertoire that is different from that in PBMC. In the lesion various V β genes seem to be dominating and the reported variation may be related to differences in the severity of the disease, or in the composition of the subgingival microbiota. The role of superantigens in periodontitis is unclear. While early reports suggested a possible influence of superantigens in periodontitis, results from recent studies questioned the validity of this hypothesis. It has been reported that a certain group of V β genes are expressed in periodontitis lesions and that such T cells respond to a restricted number of antigens.

Cytokine Profiles of Th Cells in Periodontitis

Activation of Th cells results in the production of various cytokines. Th cells are divided into Th-1 (Th-1) and Th-2 (Th-2) cells according to their cytokine profiles (Table 3). Th-1 cells produce IL-2 and IFN-γ, while Th-2 cells produce a wider range of cytokines including IL-4, IL-5, IL-6, IL-10 and IL-13. Th-1 cytokines stimulate effector T cells, i.e T cytotoxic (CD8+) cells and to some extent B cells. Th-2 cytokines, however, are in most respects directed towards B cells. As the majority of cells (about 65–70%; Fig. 2) in periodontitis lesions are B cells or plasma cells, it is reasonable to assume that Th-2 cells and related cytokines dominate over Th-1 cells in periodontitis. Results from studies evaluating cytokine profiles of Th cells in periodontitis are reported in Table 4. Only CD4+ cells were considered and the following specific search terms were used: ''*cytokine,* Th *1,* Th *2, Th-1, Th-2, IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13*''.

Fujihashi et al. (1996) collected gingival biopsies from 20 patients with adult periodontitis. CD4+ cells were isolated and reverse transcriptase polymerase chain reaction (RT-PCR) techniques were used to detect the presence of cytokines. It was reported that gingival Th cells expressed cytokines characteristic for both Th-1 and Th-2. The occurrence of IL-2 and IL-4 was rare, while IFN-γ and IL-6 were detected in CD4 cells of almost all patients. Similar findings were reported by Prabhu et al. (1996) who failed to demonstrate differences in cytokine profiles for Th-1 and Th-2 cells in gingival lesions or periph-

eral blood of periodontitis patients. Different results were reported by Tokoro et al. (1997). They used in situ hybridization-based techniques on gingival biopsies from 13 periodontitis patients and five subjects with gingivitis or healthy sites. The density of cells positive for several cytokines associated with Th-2, i.e. IL-4, IL-5 and IL-6, was higher in periodontitis specimens than in control tissues. IL-2 producing cells, however, were scarce in both periodontitis and gingivitis samples, and it was suggested that Th-2 dominates over Th-1 in periodontitis lesions. Cells identified in relation to the various cytokines in the study by Tokoro et al. (1997), however, were not tested with respect to any Th cell marker (CD4). Such markers were, on the other hand, used by Yamamoto et al. (1997). They isolated CD4+ cells from gingival tissues sampled from periodontitis patients and used RT-PCR to identify cytokine mRNA expression. While IL-2, IL-4 and IL-5 were not detected, IFN-γ, IL-6, IL-13 and to a less extent IL-10 were expressed in most cases. As only one Th-1-related and two to three Th-2-related cytokines were expressed by the isolated CD4+ cells, it was concluded that Th-2 functions dominates in periodontitis. The finding by Yamamoto et al. (1997) that no IL-4 expression was detected was in part consistent with data presented by Yamazaki et al. (1997). They collected peripheral blood samples and gingival biopsies from 17 periodontitis patients. RT-PCR was applied without a restriction to CD4+ cells. It was reported that the IL-4 expression was weak in gingival tissues. In addition, IFN-γ expression was more pronounced by PBMC than gingival tissues, while a reverse relationship was found for IL-10. The expression of IL-13 was similar in the two sample sources. The findings were interpreted to indicate a larger contribution of Th-2 than Th-1 function in periodontitis.

As reported above, the interpretation of results is in some respects difficult, and, thus, no distinct difference between Th-1 and Th-2 cytokine profiles may be distinguished in periodontitis lesions. Gemmell & Seymour (1998) collected gingival biopsies from periodontitis patients and from control subjects with healthy/gingivitis conditions. Dual-colour flow cytometry analysis revealed that there were no differences between the proportions of CD4+ cells expressing IFN-γ, IL-4 or IL-10. Gemmell et al. (1999) evaluated cytoplasmatic cytokines of *P. gingivalis*-specific T cells established from peripheral blood from periodontitis patients and healthy controls. The majority of the control subjects had higher proportion of IFN-γ+ cells, while five out of 10 periodontitis patients had higher proportions of IL-4+ and IL-10+ cells. Nakajima et al. (1999) reported that mRNA for IFN-γ and IL-13 in PBMC was up-regulated, whereas IL-4 and IL-10 expression was down-regulated following stimulation of *Porphyromonas gingivalis*. Thus, the differences with respect to enhanced or decreased expression of cytokines involved both Th-1 and Th-2 cells.

Dominance for Th-1-related cytokines in periodontitis was suggested by Takeichi et al. (2000). They isolated gingival mononuclear cells (GMC) from periodontitis patients and healthy controls. The proportion of cells expressing mRNA for IFN-γ and IL-2 was significantly higher in periodontitis than in healthy subjects and IFN-γ was the most common mRNA cytokine detected in the study. Bickel et al. (2001) collected gingival biopsies from sites representing progressive periodontitis, chronic but stable periodontitis and healthy sites. The most pronounced expression of IFN-γ was found in progressive sites, whereas the expression of IL-2 was strongest in healthy sites. Further, Gorska et al. (2003) compared cytokine expression in gingival tissues and peripheral blood, sampled from 25 periodontitis patients and 25 healthy subjects. The frequency of IL-4+ and IL-10+ samples was higher in healthy than in diseased gingival tissues, while high concentrations of IFN-γ and IL-2 correlated with severity of periodontitis.

There are also reports on a similar contribution of Th-1 and Th-2 cytokines in periodontitis. Salvi et al (1998) collected GCF, gingival biopsies and peripheral blood from eight patients with CP and from eight patients with EOP. Although Th-1 cytokines dominated over Th-2 mediators in GCF of both groups, the analysis of the limited material of mRNA expression of GMC did not favour any of the Th-1 and Th-2 cytokine profiles. Berglundh et al. (2002a, b) analysed gingival biopsies obtained from 22 subjects with severe CP. An immunohistochemical double staining technique was used to identify cytoplasmatic occurrence of Th-1- and Th-2-related cytokines in CD4+ cells. Stereologic quantification of cells positive for the different double markers was performed and no differences in the proportion of cells representing the two Th cell categories were identified. Suarez et al. (2004) investigated the expression of cytokines of T cells in gingival tissues prepared from 10 patients with aggressive periodontitis and 10 healthy controls. A constant expression of IFN-γ and IL-2 was found in all samples. IL-5 and IL-10 were found in the majority of healthy specimens but not in the samples from the periodontitis group, whereas IL-13 was only detected in the healthy sites.

Lappin et al. (2001) reported that in periodontitis lesions there were larger proportions of cells expressing IL-4 and IL-6 than cells expressing either IL-2 or IFN-γ. They analysed gingival tissues from 10 EOP and 10 AP patients and reported that IL-10 was the most frequently expressed cytokine followed by IL-6, IL-4, IFN-γ and IL-2. Further support for a Th-2 domination in periodontitis was provided by Fokkema et al. (2002), who evaluated whole blood cell cultures stimulated by bacterial lipopolysaccharides (LPS). Further, Sigusch et al. (1998) reported on a decreased production and mRNA expression of Th-1-related cytokines (IFN-γ and IL-2) following mitogen stimulation.

Summary

There are few studies using comparative designs and unbiased quantitative methods regarding Th-1 and Th-2 cells in periodontitis lesions. Further, the inability to diagnose disease activity makes interpretation of all these studies difficult. Results from direct assessments in sections and findings from analysis of isolated CD4+ cells are also conflicting. While many studies have attempted to determine the domination of either Th-1- or Th-2-related cytokines, it is highly likely that it is the balance of Th-1 and Th-2 that is important in disease expression. The relative dominance of B cells and plasma cells in periodontitis lesions cannot entirely be explained by enhanced Th-2 functions but maybe because of an inbalance in this dynamic.

Autoimmune Components in Periodontitis

The concept that autoimmune reactions are involved in periodontitis is not new. Brandtzaeg & Kraus (1965) in a study

Table 3. Cytokine profiles of T cell subsets (Th-1 vs. Th-2)

Reference	Periodontal diagnosis	Sample	Technique	Results
Wassenaar et al. (1995)	Four patients with chronic periodontitis (AAP 1989 criteria)	Biopsies from PPD $\geqslant$ 5 mm and attachment lost $\geqslant$ 4 mm	FACS analysis, cloned T lymphocytes were labelled with monoclonal antibodies (Mabs) for CD3, CD4 and CD8	Eighty percent of CD4+ T-cell clones had phenotypes resembling the mouse Th-2 phenotype (high levels of IL-4 and low levels of IFN-γ). The majority of bacterial-antigen-reactive CD4+ and/or CD8+ T-cell clones showed a Th-0-like cytokine pattern and produced equal amounts of IL-4 and IFN-γ
Fujihashi et al. (1996)	Twenty patients with adult periodontitis	Gingival biopsies and blood samples	Gingival mononuclear cells (GMC) isolated from inflamed tissues and examined by flow cytometry. Reverse transcriptase-polymerase chain reaction (RT-PCR) for detection of IFN-γ and IL-2 (Th-1), IL-4, IL-5, IL-6, IL-10 and IL-13 (Th-2)	About 20–30% of lymphocytes were CD4+ T cells. Two distinct cytokine profiles were noted. One pattern represented expression of mRNA for IFN-γ, IL-6, IL-10 and IL-13 (eight cases), the second consisted of mRNA for IFN-γ, IL-6 and IL-13 (six cases). In most samples, mRNA for IL-2, IL-4 and IL-5 were not detected. The remaining six cases did not fall into either profile. Concanavalin A-stimulated peripheral blood mononuclear cells (PBMC) showed mRNA for all Th-1 and Th-2 cytokines
Prabhu et al. (1996)	Fifteen patients with moderate to advanced periodontal diseased and two periodontally healthy subjects	Twenty-five diseased tissue samples (GTC) and 12 healthy samples. Blood samples (PBMC) from periodontally diseased and periodontally healthy	Expression of cytokine mRNA in PBMCs and GTCs was determined by PCR amplification using primers for IL-1α, IL-2, IL-4, IL-6, IL-8, IL-10, INF-α, INF-γ, TGF-β and IL-12. ELISA determined Cytokine levels in plasma	Plasma levels of Th-1 and Th-2 cytokines were below the level of detection in both patients and controls. No significant differences were detected regarding systemic mRNA levels of the cytokines between patients and controls. Differences were observed between healthy and inflamed gingival tissues in IL-6 and IFN-α mRNA expression, which was significantly higher in diseased tissues
Tokoro et al. (1997)	Thirteen adult patients with moderate-to-severe periodontitis and five patients with gingivitis or healthy periodontal tissue	Gingival biopsies	RNA probes (IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, TNF-α) for an in situ hybridization analysis	The results of single-cell analysis were used as data sets for statistical analyses. The density of cells expressing IL-1α, IL-4 and IL-5 was higher in periodontitis than in gingivitis. IL-2-expressing cells were almost absent in gingivitis specimens
Yamamoto et al. (1997)	Adult periodontitis patients (unspecified number)	Biopsies from inflamed gingival tissues (unspecified number and clinical characteristic of the sites)	Flow cytometry for isolation and characterization of CD4+ T cell subsets. RT-PCR for detection of IFN-γ and IL-2 (Th-1), IL-4, IL-5, IL-6, IL-10 and IL-13, (Th-2)	Two distinct cytokine profiles were noted. One pattern was represented by the expression of mRNA for IFN-γ, IL-6, IL-10 and IL-13, while the other case consisted of mRNA for IFN-γ, IL-6, and IL-13. Except for a few cases, messages for IL-2, IL-4 and IL-5 were not detected by cytokine-specific RT-PCR
Yamazaki et al. (1997)	Seventeen patients with moderate-to-severe adult periodontitis	Gingival biopsies (18) from sites with PPD $\geqslant$ 5 mm (mean 6.61) CAL $\geqslant$ 5 mm (mean 8.89) mean bone loss 68.33%, Bop+and peripheral blood samples	RT-PCR and subsequent image analysis was used to determine the level of mRNA for each cytokine (INF-γ, IL-4, IL-10, IL-12 p35, IL-12 p40, IL-13	The mean expression of IFN-γ mRNA was significantly higher in PBMCs than in gingival tissues. In contrast, the mean expression of IL-10 mRNA was higher in gingival tissues than in PBMCs. There was no difference in the mean expression of IL-13 in gingival tissues and PBMCs. IL-4 mRNA was weak but detectable in three gingival tissue samples

Gemmell & Seymour (1998)	Thirty-one subjects with adult periodontitis (AP) and 31 individuals forming healthy/gingivitis group (H/G)	Gingival biopsies; 15 AP and 13 from H/G subjects for T-cell cytokines and CD30; 18 AP and 14 H/G for IL-1β and IL-10 by B cells and Mϕ; 16 AP and 12 H/G for B cell and Mϕ IL-12. Peripheral blood samples from nine *Porphyromonas gingivalis* +AP and 10 H/G subjects	Immunohistochemistry and dual-colour flow cytometry on a FACS analyzer to determine the percent IFN-γ-, IL-4-, IL-10-, and CD30-positive T-cells and the percentages of IL-1 β-, IL-10- and IL-12-producing B cells and macrophages	No significant differences between the percent IL-4+or INF-γ+CD4 and CD8 cells or IL-10+CD4 cells extracted from AP or H/G lesions. The percent IL-10+CD8 cells extracted from AP lesions was decreased compared with H/G tissues. The percentage CD30+CD4 and CD30+CD8 cells was higher in AP than in H/G tissues. The percentage of IL-1 β+macrophages and IL-12+B-cells were both higher in AP lesions than in H/G tissues
Salvi et al (1998)	Eight patients with adult severe periodontitis (AP) and eight patients with generalized early-onset periodontitis (EOP). All patients were selected on the basis of possessing a terminal dentition (TD)	Peripheral blood for monocytes isolation and culturing, gingival crevicular fluid (GCF) and gingival biopsies from each patient	ELISA for cytokines GCF determination. ELISA evaluated concentration of PGE2, IL-1β and TNF-α from monocytes in peripheral blood. GMC and T cell subsets were isolated from gingival biopsies and by RT-PCR, the expression of cytokines (INF-γ, IL-2, IL-4, IL-5, IL-6, IL-1β, TNF-α, IL-12, IL-10, IL-13, TGF-β) were determined	The GCF analysis clearly indicated that in both AP and EOP groups the monocyte, i.e. IL-1 β and PGE2 and Th-1, i.e. IL-2 and IFN-γ, inflammatory mediator levels quantitatively dominated over the Th-2 mediators, i.e. IL-4 and IL-6. Tissue samples were too small to be analysed for mRNA expression. four AP and six EOP samples for mononuclear cells and three AP and three EOP for the T cells were recovered. Neither group of patients favoured a Th-1 or Th-2 cytokines expression. However CD4+T cells demonstrated a good expression of INF-γ, IL-6 and IL-10
Sigusch et al. (1998)	Eleven patients with adult periodontitis (AP), 14 patients with EOP and 20 control subjects.	Peripheral blood samples	PBMC isolated and stimulated. Immunofluorescence to determine lymphocyte subsets (CD3, CD4, CD8, CD14, CD57) and HLA-DR expression on monocytes. ELISA to measure levels of IL-3, IL-5, GM-CSF and IFN-γ. Cytokine mRNA expression in PBMCs determined by PCR.	PBMC in EOP patients expressed significantly decreased levels of IFN-γ in response to mitogenic stimulation. Reduced IFN-γ secretion was associated with decreased IFN-γ and IL-2 mRNA expression in these cells, as well as decreased HLA-DR surface expression on monocytes. On the other hand, it was observed a significantly higher level of IL-5 and GM-CSF using PBMC from AP patients.
Nakajima et al. (1999)	Four subjects with adult periodontitis and five healthy or gingivitis individuals	Peripheral blood samples and plaque sample from each subject	Monoclonal antibodies and ELISA to detect *P. gingivalis* in plaque and blood samples. Cytokine (IL-4, INF-γ, IL-10, IL-12p35, IL-12p40, IL-13) mRNA expression was evaluated by RT-PCR	Messenger RNA for IFN-γ and interleukin-13 was up-regulated whereas IL-4 and IL-10 were down-regulated following *P. gingivalis* stimulation. Interleukin-12p35 demonstrated no consistent pattern
Gemmell et al. (1999)	Ten subjects with adult periodontitis and seven	Peripheral blood samples	Flow cytometric analysis to determine the percentage of CD4+ and CD8+ T cell	There were no differences in the mean percentage of IL-4-, IFN-γ- or IL-10-positive T cells between the two groups. However, the individual profiles showed that the CD4 cells in five of the seven healthy or gingivitis lines had

Table 3. (Contd.)

Reference	Periodontal diagnosis	Sample	Technique	Results
	healthy or gingivitis individuals		lines staining positive for cytoplasmatic IL-4, INF-γ and IL-10	a higher proportion of IFN-γ -positive cells, with two lines demonstrating higher percentage of IL-10- and/or IL-4-positive CD4 cells. Five of the 10 adult periodontitis lines demonstrated either equal or higher percentages of IL-4-positive and/or IL-10-positive CD4 cells
Takeichi et al. (2000)	Fifty-three patients with chronic adult periodontitis (bone loss and PPD >5 mm) and eight controls with no bone loss and PPD $\leqslant 3$ mm	Gingival biopsies (37) from adult periodontitis were used 25 for the GMC isolation and analysis and 12 for T lymphocytes analysis. Tissue biopsies from controls	The expression of mRNA for IL-2, IL-5, IL-6 and IFN-γ by GMC by using cytokine-specific RT-PCR	Significantly higher GMC proportions from AP patients expressed IL-2 and IFN-γ mRNA than those from healthy subjects. AP gingival T-lymphocytes expressed mRNA for IL-2, IFN-γ, or IL-6 prior to stimulation. After stimulation with Con A, the cells significantly up regulated IL-5 and IL-6 message expression. Both CD4+ and CD8+ gingival T-lymphocytes expressed IFN-γ, IL-5, and IL-2
Bickel et al. (2001)	Six patients diagnosed as severe localized 'refractory' periodontitis, each presenting sites affected with (1) severe progressive periodontitis, (2) chronic, but stable periodontal lesions and (3) with healthy sites	Gingival biopsies: healthy sites PPD from 3 to 4 mm), stable PPD from 5 to 7 mm (no change at two different measurement), progressive PPD from 5 to 10 mm (increased PPD $\geqslant 2$ mm at different intervals)	The biopsies analysed for levels of cytokine mRNAs. Analysis using a quantitative RT-PCR included IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, and TNF-α	Results revealed variation not only between patients but also between individual sites. In general, expression of IFN-γ was highest in progressive lesions, while the healthy sites did not differ from the stable lesions. IL-2 was expressed stronger in healthy sites than in stable and progressive sites. IL-4 showed an increase from healthy to stable and to progressive sites
Lappin et al. (2001)	Ten patients with EOP and 10 patients with adult periodontitis (AP)	Biopsies from AP with PPD ranged from 6 to 9 mm; and from EOP with PPD ranged from 6 to 10. Ten control (healthy sites) biopsies from the adult periodontitis patients	Immunohistochemistry to detect cells expressing IL-2, IL-4, IL-6, IL-10 and IL-15, TNF-α and IFN-γ in sections from periodontitis lesions. In situ hybridization for mRNA detection	Cells that expressed IL-4 or IL-6 were more numerous than cells expressing either IL-2 or IFN-γ. Th-2 cells were more numerous in EOP and AP tissues. The most widely expressed cytokine in the tissue was IL-10. This was followed by IL-6, IL-4, INF-γ and IL-2
Berglundh et al. (2002a, b)	Twenty-two adult patients with advanced and generalized chronic periodontitis, exhibiting bone loss $>50\%$ at all the teeth and PPD >6 mm at 80% of the proximal sites	Gingival biopsy from each patient from randomly selected diseased interproximal site with PPD >6 mm and Bop+	Immunohistochemistry. Double staining was performed to detect cells positive for both the CD4 marker and different cytokines, i.e. IL-2, IL-4, IL-6 and IFN-γ	The lesions in advanced periodontitis contained similar proportions of cells positive for the different cytokine markers examined. In addition, the number of cells expressing cytokine profiles for either T helper-1 (IFN-γ+IL-2) or T helper-2 (IL-4+IL-6) was similar
Fokkema et al. (2002)	Nineteen patients with chronic untreated periodontitis (bone loss $>1/3$ of the total length of the root on two or more teeth per quadrant) and 19	Peripheral blood samples	Whole blood cell cultures (WBCC) were stimulated with LPS from *Escherichia coli* and the release of PGE2, IL-1β, IL-6, IL-8, IL-10, IL-	The levels of PGE2 were twofold higher in the WBCC from periodontitis patients than from controls. In contrast, the levels of IL-12p70 in WBCC from patients were twofold lower. Furthermore, WBCC from patients secreted lower levels of IL-1β and higher levels of IL-8 when compared

	periodontally healthy control subjects		12p40, IL-12p70 and TNF-α was measured by ELISA and by ACE (competitive enzyme immunoassay) for PGE_2 concentrations	with WBCC from controls. No differences were observed with respect to IL-6, IL-10, IL-12p40 and TNF-α production
Gorska et al. (2003)	Twenty-five patients with severe chronic periodontitis, who had sites with probing depths (PPD) >5 mm at least at four sites and 25 periodontally healthy (PPD $\leqslant 3$ mm) subjects	Gingival tissue biopsies were collected from sites with PPD >6 mm and Bop+of each patient and from healthy individuals Blood samples were obtained on the day of tissue biopsy	The concentrations of cytokines (IL-1β, TNF-α, IL-2, IFN-γ, IL-4, IL-10) were determined by an enzyme-linked immunosorbent assay (ELISA)	The concentrations of IL-1β, TNF-α, IL-2, and IFN-γ were, on average, significantly higher in serum samples and gingival tissue biopsies from periodontitis patients than in healthy controls. The frequency of IL-4 (88% positive samples) and IL-10 (72%) was higher in healthy gingival tissues. High concentrations of TNF-α, IFN-γ and IL-2 and high ratios of IL-1β/IL-10 and TNF-α/IL-4 found in tissue biopsies from periodontitis patients, strongly correlated with the severity of periodontitis
Suarez et al. (2004)	Ten patients with aggressive periodontitis (AgP) and 10 periodontally healthy individuals with gingival index of 1 (HI/G1)	Ten gingival samples of HI/G1 individuals and 10 gingival samples of AgP patients from site with Bop+and PPD >6 mm	Immunohistochemistry for detection of CD3+, CD4+ and CD8+ cells. The presence of IL-2, IFN-γ, IL-4, IL-5, IL-10, IL- 13 and TGF-β was measured by RT-PCR of mRNA extracted from complete gingival biopsies	The % of CD3+ in relation to the total amount of mononuclear cells was lower in patients with AgP. The CD4+/CD8+ ratio was reduced in patients with AgP when compared to healthy. The cytokine mRNA analysis showed constant expression of IL-2 and IFN-γ in all cases. The mRNA of IL-5 and IL-10 was present in the majority of HI/G1 but was not in the AgP group. IL-13 and TGF-β were only detected in HI/G1 and IL-4 was not detected in any of the individuals

entitled ''Autoimmunity and periodontal disease'' reported on the presence of antibody producing plasma cells in periodontitis lesions. In later reports the presence of auto-antibodies, auto-reactive B cells and other autoimmune components in periodontitis were described. Publications related to the field are presented in Table 4. The specific search terms added were ''*autoimmunity, auto-antibody, auto-reactive, anti-collagen B-1cells, CD5, IgG, IgM and plasma cells*''.

Anti-collagen antibodies and cells

A common target for analysis of autoantigens was collagen type I. Ftis et al. (1986) collected peripheral blood from 97 periodontitis patients and 57 controls. The level of antibodies to collagen type I was significantly higher in the periodontitis patients than in the controls. In contrast to this observation, Hirsch et al. (1988) in a study on 39 periodontitis patients reported that anti-collagen producing cells were rarely detected in peripheral blood and levels of anti-collagen antibodies in serum were low. Hirsch et al. (1988) also isolated gingival cells and found that cells forming specific antibodies to type I collagen were numerous in relation to the total number of antibody-producing cells. Corroborating data were presented by Jonsson et al. (1991), who detected anti-collagen type I-antibody secreting cells in gingival tissues obtained from 15 patients with CP, and by Anusaksathien et al. (1992) and Rajapakse & Dolby (2004) who found that the level of antibodies to collagen type I was higher in gingival tissues than in peripheral blood. Anusaksathien et al. (1992) further reported that both IgG and IgA were found in higher concentrations in gingival tissue extracts than autologous serum, while no differences were found regarding IgM levels. The authors suggested that anti-collagen antibodies in gingival tissues undergo class-switch from IgM to IgG.

GCF was also collected from periodontitis sites for the detection of antibodies to collagen type I. Sugawara et al. (1992) compared anti-collagen IgG levels in GCF and in serum in 20 periodontitis patients. It was reported that the IgG levels in GCF were slightly higher than that in autologous sera and significantly higher compared with levels in sera collected from healthy control subjects. Other components of the immune

Table 4. Auto-immune components in periodontitis

Reference	Periodontal diagnosis	Sample	Technique	Results
Ftis et al. (1986)	Ninety-seven patient with periodontal disease (Russell Periodontal Index 1.0–7.0) and 57 control subjects (PI < 1)	Blood samples	Enzyme-linked immuno-sorbent assay (ELISA)	Levels of antibody to type I collagen detected in patients were higher ($p < 0.001$) than in control subjects
Hirsch et al. (1988)	Thirty-nine adult periodontitis patients (AP)	Thirty-nine biopsies and peripheral blood samples	Enzyme-linked immunospot (ELISPOT) test	Biopsies revealed the presence of high numbers of cells that secrete antibodies to Type I collagen and, to a lesser extent, Type III. The majority of such cells produced specific antibodies of the IgG class, but IgA- and IgM-secreting cells were also detected. Anti-collagen antibody-producing cells were rare and levels of anti-collagen antibodies low in blood of AP patients
Jonsson et al. (1991)	Fifteen ''untreated'' and 16 patients scheduled for treatment of chronic adult periodontitis	Biopsies at tooth extraction for untreated patients and at surgery-time (after SRP) for the treatment subjects	Production of antibodies to collagen type I was analysed by means of an ELISPOT assay	The frequency of immunoglobulin secreting cells was 1.9 ± 1.9% of the recovered mononuclear cells in untreated and 1.1 ± 1.0% in the treatment subjects. In the first group the anti-collagen antibodies were most often of IgG and IgM isotype. In the treatment group, IgA and IgM isotype predominated, while IgG was an infrequent finding
Afar et al. (1992)	Periodontitis patients (18) with 5–25% bone loss and 5–10 mm PPD. Sixteen periodontally healthy control subjects	Venous blood samples from test and control subjects	Flow cytometry and mono-clonal antibodies	The frequency of CD4+ and CD5+T cells, CD20+B cells, and CD16+NK cells were increased in periodontitis patients. CD4+ activated ''memory'' T cells, CD5+B cells and CD56+NK effector cells were significantly increased in periodontitis patients
Anusaksathien et al. (1992)	Fifteen periodontitis patient (Russell Periodontal Index 1.8–6.0)	Biopsies (PPD 5–10 mm) and blood samples	ELISA (Ig/albumin ratio in tissue and serum)	IgG and IgA were present in higher concentration in tissue extracts than in autologous serum when adjustment was made for dilution differences. No significant differences were found for IgM antibodies.
Sugawara et al. (1992)	Twenty periodontitis patients and 10 periodontally healthy controls	From periodontitis patients: 10 GCF for anti-collagen antibodies, 20 biopsies and 20 blood samples. From healthy subjects: 10 blood samples	Flow cytometric analysis, immunohistochemical analysis, ELISA for detection of anti-collagen antibody in GCF and sera	The percentage of CD5+B cells was statistically higher in gingiva than in peripheral blood in both the patients and controls. Anti-collagen IgG antibody levels in GCF of patients was higher than that in sera from healthy subjects, and slightly higher than in autologous sera. CD5+B cells produced considerably more IgM and IgG antibodies to collagen than CD5 − B cells.
Govze & Herzberg (1993)	Ten periodontitis subjects (evidence of bone loss and PPD ⩾ 4 mm; GI ⩾ 1 mm); 10 control subjects	Blood samples and GCF samples.	SDS polyacrylamide gel electrophoresis for isolation of desmosomal proteins. Western Immunoblot technique to reveal reactions of desmosomal proteins and glycoproteins to anti-bodies in serum and GCF	Sera from 90% of subjects with periodontitis showed increased reactions of IgG with desmoplakins and 80% with desmogleins, when compared to controls (10%). When GCF within individuals with periodontitis was compared, anti-desmosomal IgG from diseased sites showed greater reactivity than healthy sites and control patients
Wassenaar et al. (1995)	Four patients with chronic periodontitis (AAP 1989 criteria)	Biopsies from PPD ⩾ 5 mm and attachment lost ⩾ 4 mm	FACS analysis, cloned T lymphocytes were labelled with monoclonal antibodies (Mabs) for CD3, CD4 and CD8	CD4+T-cell clones reactive with collagen type I were obtained from all patients. 80% of these clones had phenotypes resembling the T helper 2-phenotype. No collagen-type-I-reactive CD8+ clones were obtained

Aramaki et al. (1998)	Thirty-four adult periodontitis patients (with moderate-to-severe bone loss at more than one tooth) and 10 periodontally healthy blood donors as controls	PBMC were obtained from 29 AP peripheral blood samples and 10 controls. Gingival mononuclear cells (GMC) were recruited from 15 diseased and healthy sites of AP patients	ELISA for quantification of Ig and cytokines (IL-6 and IL-10). Two-colour flow cytometry for counting CD20+ and CD20+ CD5+	The proportion of B-1a cells and the amount of IL-6 and IL-10 were significantly higher in the inflamed gingival tissues than in peripheral blood from the healthy subjects
Novo et al. (1999)	Thirty SLE subjects (perio + and perio-) and 30 RA patients (perio+ and perio −). Healthy subjects (no SLE, RA or Perio) as a controls (20)	Blood samples from each patient of all the categories	Indirect ELISA assay testing sera for the presence of antibodies directed to a Neutrophil granular extract and the granular enzymes	A high number of ANCA-positive sera in SLE were found mostly in individuals presenting periodontal disease. A statistically significant association between ANCA and periodontitis in SLE patients was found ($p<0.005$, chi square test)
Tabeta et al. (2000)	Moderate to advanced periodontitis patients (23) and 18 periodontally healthy individuals as controls	Blood samples (test and control subjects) and gingival tissue samples (10 from periodontitis) to evaluate antibodies to heat shock protein 60 (hsp60) and to *Porphyromonas gingivalis* heat shock protein (GroEL)	Western blot analysis of sera and gingival tissue extract and ELISA for anti-human hsp60 and anti-*P. gingivalis* GroEL	For human hsp60, a higher frequency of seropositivity was found in the periodontitis patients than in the healthy subjects. Quantitative analysis of serum antibodies (anti-hsp60) demonstrated significant higher levels in patients than those of control subjects. In the gingival tissue extracts, seven out of 10 patients demonstrated a positive response to human hsp60 and two of these demonstrated strong positivity.
Sims et al. (2001)	Eighteen subjects diagnosed with IDDM and the following periodontal conditions: Bop+ at >8 sites, CAL $\geqslant$5.0 mm on four or more non-adjacent teeth	Blood samples (and clinical data) before and 2 months after SRP to evaluate serum glutamic acid decarboxylase autoantibody GAD Ab levels	Radioligand precipitation and ELISA to detect respectively GAD Ab and IgG to P. gingivalis (Pg), Bacteroides forsythus (Bf) and Aa	The decrease of PPD was significantly better for GAD Ab-seronegative subjects than for seropositive subjects. GAD Ab levels and PPD were positively correlated ($p<0.05$) for serum-positive subjects but were neutral for serum-negative subjects. Serum IgG to Pg and GAD Ab levels were positively associated in serum-positive subjects
Berglundh et al. (2002a, b)	Advanced and generalized chronic periodontitis (21), localized aggressive periodontitis (7) and periodontally healthy subjects (26).	Blood samples form all three categories. Biopsies from diseased sites in chronic periodontitis subjects	Immunohistochemistry (CD5+ and CD19+ cell markers), Flow cytometry	About 40–50% of the B cells in the peripheral blood of the periodontitis susceptible individuals expressed markers for autoreactive features while less than 15% of the circulating B cells in the healthy subjects exhibited such markers. The periodontitis lesion in AP patients contained a substantial number of B cells out of which about 30% demonstrated autoreactive features.
Schenkein et al. (2003)	Periodontally healthy (NP) subjects (163); Localized aggressive Periodontitis (LagP) patients (32); Generalized aggressive periodontitis (GagP) patients (87) and 129 Chronic periodontitis subjects (CP)	Blood samples for determination of IgG and IgM anti-cardiolipin (anti-CL) and IgG anti-β-2-glycoprotein-I (anti-β2 GP-I)	ELISA	The prevalence of patients with chronic periodontitis (CP) and generalized aggressive periodontitis (GAgP) positive for anti-CL was greater than that in healthy controls (NP) and localized aggressive periodontitis (LAgP) patients. Analysis of the data indicates that patients with generalized periodontitis have elevated levels of autoantibodies reactive with phospholipids

Table 4. (Contd.)

Reference	Periodontal diagnosis	Sample	Technique	Results
Rajapakse & Dolby (2004)	Thirteen periodontitis patients and age- and sex-matched periodontally healthy subjects as controls	Biopsies (15) at diseased sites (PPD $\geqslant$6mm) and blood samples to evaluate autoantibodies to collagen Type I, and antibodies to *P. gingivalis*, *Actinobacillus actinomycetemcomitans* and *Bacteroides fragilis*	ELISA for detection of antibody to collagen and *A. actinomycetemcomitans*, *P. gingivalis* and *B. fragilis* in tissue elutes and serum.	Level of antibodies to collagen type 1 in tissue was significantly higher than in serum ($p = 0.0001$). Antibody levels in tissue to *P. gingivalis* were significantly higher than in serum ($p = 0.0271$). Ab levels to both *A. actinomycetemcomitans* and *B. fragilis* in tissues and serum were not significantly different from each other
Yamazaki et al. (2004)	Twenty-one patients with moderate to advanced chronic periodontitis	Blood samples to evaluate levels of antibody to human hsp60 before and after periodontal treatment	Antibody levels were determined using an enzyme-ELISA.	The mean level of anti-human hsp60 antibody remained unchanged although individual levels of antibody either increased or decreased after periodontal treatment, suggesting that synthesis of these antibodies might be regulated independently during the course of periodontal infection

systems than antibodies have also been associated with anti-collagen characteristics. Wassenaar et al. (1995) analysed T cells isolated from periodontitis lesions. While Th (CD4+) cells were found to be reactive with collagen type I, no T cytotoxic (CD8+) cell clones were found to have anti-collagen characteristics. In addition, about 80% of the CD4+ cell clones exhibited the Th 2-phenotype.

Auto-antibodies to cellular components

Although anti-collagen reactions dominate in studies on autoimmunity and periodontitis, additional targets for analysis were used. Govze & Herzberg (1993) analysed auto-antibodies to desmosomal proteins in GCF and peripheral blood from 10 periodontitis patients and 10 controls. The sera from the majority of periodontitis patients showed increased reactions of IgG to desmosomal proteins in comparison with sera from controls. Further, anti-desmosomal IgG in GCF sampled from diseased sites demonstrated greater reactivity than that of healthy sites. Another target for analysis was anti-neutrophil cytoplasmatic antibodies (ANCA). Novo et al. (1999) recruited two groups of subjects with autoimmune disorders; 30 subjects with systemic lupus erythematosus (SLE) and another 30 subjects with RA. Both groups were divided into periodontitis and non-periodontitis subjects. The amount of ANCA in the RA group did not differ between patients with and without periodontitis, while in the SLE group significantly greater values of ANCA were found in periodontitis patients. The authors suggested that the general elevated levels of different auto-antibodies in SLE patients together with an enhanced B cell activation in periodontitis may explain the observed differences between the groups. Another group of auto-antibodies frequently found in SLE patients is anti-phospholipids. Schenkein et al. (2003) examined anti-phospholipid antibodies in blood samples from 163 healthy subjects and from 32 patients with localized aggressive periodontitis (LagP), 37 patients with generalized aggressive periodontitis (GagP) and 129 CP patients. Larger proportions of subjects positive to anti-phospholipid antibodies were found in GagP and CP groups than in LagP and healthy subjects. It was suggested that generalized forms of periodontitis are associated with anti-phospholipid antibodies and that such antibodies may be involved in the elevated risk for cardiovascular disorders in periodontitis subjects.

B-1a cells

A particular group of cells in autoimmune reactions are CD5+B cells, termed B-1a cells (Kantor 1991). This group of B cells is found in large numbers in the peripheral blood of patients with autoimmune diseases, e.g. RA and Sjögren's syndrome (Burastero et al. 1988, Youinou et al. 1988) and produces IgM auto-antibodies as well as antibodies to bacterial antigens, such as LPS. While conventional B (B-2) cells are developed from bone marrow precursors, B-1a cells are developed from peritoneal precursor cells. Further, B-1a cells may develop into plasma cells and produce immunoglobulins of other classes than IgM.

The presence of B-1a cells in periodontitis patients have been demonstrated in several reports. Afar et al. (1992) collected blood samples from 18 patients with varying severity of periodontitis and from 16 healthy control subjects. Flow cytometry analysis revealed that B-1a cells occurred in significantly larger amounts in periodontitis patients than in controls. Similar results were reported by Berglundh et al. (2002a, b). They analysed B-1a cells in peripheral blood of three different groups of subjects; 22 subjects with severe generalized CP, seven children with localized aggressive periodontitis and 26 healthy controls. The proportions of B-1a cells were five to six times greater in the periodontitis groups than in the controls and it was stated that up to 40–50% of all circulating B (CD19+) cells were positive to the additional marker of CD5, i.e. the characteristics of B-1a cells. In this context it is interesting to note that systemic levels of B-1a cells seem to be a marker of susceptibility to periodontitis rather than an indicator of the presence of the disease (Berglundh et al. 1999). Berglundh et al. (1999) in a study on local and systemic features of host response in CP before and after non-surgical periodontal therapy, found that the elevated numbers of B-1a cells did not decline after therapy despite the sufficient clinical signs of healing.

Large amounts of B-1a cells were detected in the periodontitis lesions of CP patients in the study by Berglundh et al. (2002a, b). This observation was in agreement with earlier findings by Suga-

wara et al. (1992) and Aramaki et al. (1998). They found larger amounts of B-1a cells in the gingival lesions than in peripheral blood and suggested that B-1a cells are activated in periodontitis lesions. Aramaki et al. (1998) further reported that IL-10, which is an autocrine growth factor for B-1 cells, was also found in higher levels in gingival tissues than in peripheral blood. Increased levels of IL-10 together with large proportions of B-1a cells was also reported in a study on type 1 diabetics by Stein et al. (1997). It was suggested that periodontal pathogens induce a hyperactive IL-10 response leading to proliferation of B-1a cells and to autoantibody production. The relationship between autoimmune components in type 1 diabetics and periodontitis was also investigated by Sims et al. (2001). Patients who were sero-negative for glutamic acid decarboxylase auto-antibodies (GAD Ab) had better outcomes following non-surgical periodontal therapy than patients that were GAD Ab-sero-positive.

Hsp60

A recently demonstrated target for autoimmune reactions in periodontitis is hsp60. Hsp60 is considered to be highly immunogenic and has a bacterial homologue, GroEL. Tabeta et al. (2000) in a study on 23 periodontitis patients and 18 controls reported that there was a higher frequency of seropositivity to hsp60 in the periodontitis group than in the control group. Gingival tissue samples prepared from 10 of the periodontitis patients were in 70% of cases also positive to hsp60. Yamazaki et al. (2004) analysed antibodies to hsp60 in blood samples from 21 patients with moderate to advanced CP before and after periodontal therapy. It was reported that the hsp60 antibodies remained unchanged during treatment and the authors suggested that the synthesis of these antibodies is regulated independently during the course of periodontal infection.

Summary

As in other chronic infectious diseases, several components related to autoimmune reactions also occur in periodontitis lesions and involve specific cell groups as well as antibodies to collagen type-1 and other tissue or cell products. However, the role of autoantibodies in the regulation of host response in periodontitis as in other chronic infectious diseases needs to be clarified. There is convincing evidence that auto-reactive B cells, i.e. B-1a cells, occur in larger proportions in subjects with chronic and aggressive forms of periodontitis than in healthy controls. Results from one study indicate that the elevated numbers of B-1a cells may illustrate a feature of susceptibility rather than the presence of disease. A substantial proportion of B cells in periodontitis lesions are B-1a cells. The enhanced levels of this cell group in periodontitis are associated with increased levels of IL-10, which is considered to be an autocrine growth factor for B-1a cells.

Other components, such as hsp60, may also contribute to activation of autoimmune reactions in periodontitis and, in this context, may also represent a link between periodontal infections and systemic diseases.

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Address:
Tord Berglundh
Department of Periodontology
The Sahlgrenska Academy at Göteborg University
Box 450
SE-405 30 Göteborg
Sweden
E-mail: berglundh@odontologi.gu.se